

**REMARKS**

Claims 62-78 are pending. Claims 62-78 are pending. Claim 62, 63, 69, 72 and 76-78 are amended, as discussed below.

It is respectfully submitted that the present amendment and response presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

**I. SPECIFICATION**

The Examiner contends that the specification is still "somewhat confusing in the recitation 'may also be separated as follows, i.e., meaning the same as the plus sign Asp30Ala/Ser34Glu or N30A/S34E' as applicants have not maintained consistency with the first part of applicants amendment which recites 'Ala30Asp + Glu34Ser or A30D+E34S.'"

This rejection is respectfully traversed. The referenced paragraph clearly states that the recitation Ala30Asp + Glu34Ser or A30D+E34S (which use a plus "+" sign between the mutations) is equivalent to the recitation Asp30Ala/Ser34Glu or N30A/S34E (which use a slash "/" between the mutations). There is nothing confusing in this paragraph and the Examiner's statement that the two recitations lack consistency is not valid. The two phrases are simply suitable alternative recitations.

In the Advisory Action, the Examiner states that while the previous amendment was considered to overcome the objection, the Examiner still finds the recitation confusing for lack of consistency. The Examiner's comments are confusing. If the Examiner still believes the language is confusing, Applicants respectfully request that the Examiner provide a suggestion to clarify the language as Applicants do not see any inconsistency or confusion. If the Examiner believes the language is not confusing, Applicants respectfully request that the Examiner withdraw the objection.

**II. Claim Objections**

Claims 63, 69 and 76-78 are objected to as follows:

Claim 63 contains a superfluous "that." Applicants appreciate the Examiner's identification of this issue and claim 63 has now been amended to correct the duplicate use of the term "that."

The Examiner states that claims 63 and 73-78 further limit the claim from which they depend and should accordingly state "further comprising" instead of "comprising."

With respect to proposed use of the recitation "further comprising," the term "comprising" in claim 62 and in claim 72 means that the claim is open to other alterations. Furthermore, a dependent claim, by definition, includes all of the limitations from the claim from which it depends. Thus, it is not clear why the term "further" is necessary or would add any more clarity since the dependent claims define the same invention with the transitional phrase "comprising" as they do with the transitional phrase "further comprising." Nevertheless, in order to expedite prosecution, the claims are amended as requested by the Examiner.

Applicants respectfully request reconsideration and withdrawal of the objection.

### III. The Rejection of Claims 62-78 under 35 U.S.C. 112

Claims 62-78 are rejected under 35 U.S.C. 112, first paragraph, as allegedly lacking enablement. The Examiner alleges that a skilled artisan is not enabled to prepare nucleic acids encoding alpha-amylases having an amino acid sequences which are at least 70% identical to SEQ ID NO:4, at least 80% identical to SEQ ID NO:4, at least 90% identical to SEQ ID NO:4, or at least 95% identical to SEQ ID NO:4 and which comprise an alteration at a position corresponding to position 356 or 376 in SEQ ID NO:4. The Examiner also contends that the artisan would apparently have to make and test an infinite number of nucleic acid sequences in order to practice the invention in a manner reasonably correlated with the scope of the claims.

In the Advisory Action, the Examiner states that Applicants arguments are not persuasive because while the methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, were well-known to the skilled artisan, producing such variants as claimed requires that one of ordinary skill in the art know or be provided with guidance for the selection of the infinite number of variants having the claimed property. The Examiner alleges that without such guidance, one of ordinary skill in the art would be reduced to producing and testing virtually infinite possibilities. The Examiner alleges that the specification does not establish (A) regions of the protein structure which may be modified without effecting alpha-amylase activity; (B) the general tolerance of alpha-amylases and the extent of such tolerance; (C) a rationale and predictable scheme for modifying any amino acid residue of any alpha-amylase with an expectation of obtaining the desired biological function and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Applicants respectfully submit that the Examiner's reasons for maintaining the enablement rejection are based on a state of art that is far from indicative of what the actual

state of art was at the time of the invention. Moreover, although the Examiner alleges that an artisan would have to produce an essentially infinite number of variants to be enabled to practice the claimed invention, the Examiner has not explained why this is required. Finally, the Examiner also does consider that the state of the art available at the time of the invention permitted an artisan produce and screen essentially an infinite number of variants, thus, the Examiner's rejection is based on an incorrect factual assumption.

Section 112 of U.S. Patent Code requires that the specification be "enabling" to a person skilled in the art to which the invention pertains. "A specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of section 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." *In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971).

It is also well settled that an assertion by the Patent Office that the enabling disclosure is not commensurate in scope with the protection sought must be supported by evidence or reasoning substantiating the doubts so expressed. *In re Dinh-Nguyen*, 181 U.S.P.Q. 46 (C.C.P.A. 1974). See also *U.S. v. Telecommunications*, 8 U.S.P.Q.2d 1217 (Fed. Cir. 1988); *In re Bowen*, 181 U.S.P.Q. 48 (C.C.P.A. 1974); *Ex parte Hitzeman*, 9 U.S.P.Q.2d 1821 (BPAI 1988). In the absence of any evidence or apparent reason why compounds do not possess the disclosed utility, the allegation of utility in the specification must be accepted as correct. *In re Kamal*, 158 U.S.P.Q. 320 (C.C.P.A. 1968). See also *In re Stark*, 172 U.S.P.Q. 402, 406 n. 4 (C.C.P.A. 1972) (the burden is upon the Patent Office to set forth reasonable grounds in support of its contention that a claim reads on inoperable subject matter).

Enablement requires that the specification teach those in the art to make and use the invention without undue experimentation. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). As stated in *Wands*, [w]hether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." See *id.* at 1404. The *Wands* factors which may be relevant for determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. *Id.*

Claims 62 and 72 recite the structural feature that the variant alpha-amylase comprises an amino acid sequence which is at least 70% homologous to the amino acid sequence of SEQ ID NO: 4. Claims 64 and 73 recite the structural feature that the variant alpha-amylase comprises an amino acid sequence which is at least 80% homologous to the amino acid sequence of SEQ ID NO: 4. Claims 65 and 74 and recite the structural feature that the variant alpha-amylase comprises an amino acid sequence which is at least 90% homologous to the amino acid sequence of SEQ ID NO: 4. Claim 66 and 75 recite the structural feature that the variant alpha-amylase comprises an amino acid sequence which is at least 95% homologous to the amino acid sequence of SEQ ID NO: 4.

For decades the scientific community has relied on the structural features of percent identity of amino acid sequences to reasonably predict the function of polypeptides and to place the polypeptides and the nucleic acids that encode them into an existing genus. In particular, polypeptides which have a high degree of sequence identity to another polypeptide are expected to have a very similar function. The United States Patent Office and patent authorities throughout the world have also accepted these structural features to define a genus of polypeptides.

The structural feature of 70% identity to a reference sequence provides a reasonable prediction of relatedness of members of the genus. An alpha-amylase which has at least 70% identity to a reference alpha-amylase will share a very similar amino acid sequence, differing from only 1 amino acid up to only 30% of the amino acids, such that the majority (at least 70%) of amino acids are identical to the reference sequence. Based on the high degree of identity between alpha-amylases which have amino acid sequences which are at least 70% identical, such alpha-amylases will also have a very similar three-dimensional structure, that is, the amino acids will associate in a very similar fashion (e.g., formation of hydrophobic areas, formation hydrophilic areas, electrostatic interaction, etc.) to form a three dimensional structure that very much resembles the three dimensional structure of the reference sequence. Indeed, the majority of the structural areas of the alpha-amylase will be virtually identical to the reference sequence. Polypeptides which have an even higher degree of identity, in particular, 80%, 90%, and 95%, provide even great structural similarity.

Accordingly, when alpha-amylases share a very similar primary structure, an artisan can reasonably expect that modifications which are applicable to the reference sequence will be applicable to other members of the defined genus. In this regard, the claims are narrowly drawn to encompass a family of alpha-amylases (those which have at least 70% identity to SEQ

SEQ ID NO:4) which an artisan can expect with a reasonably high degree of certainty that the claimed alterations will apply.

The Examiner's rejection primarily focuses on the alleged unpredictability in which amino acids can be modified (i.e., up to 30%) without impacting the alpha-amylase activity and how one skilled in the art would carryout such tasks without undue experimentation. The Examiner alleges that there is not sufficient guidance on which amino acids must be conserved and which can be altered. The Examiner bases this allegation on the alleged absence of guidance of the general tolerance of the alpha-amylase enzyme to alterations, appropriate alterations which can be made, amino acids/structure which must be conserved, a rational and predictable scheme for modifying amino acid residues to obtain the desired biological function and which of the "virtually infinite" possible choices is likely to be successful. The Examiner concludes that an artisan is left with undue experimentation in producing and testing virtually infinite possibilities.

The Examiner's rejection is based on incorrect factual assumptions and an improper application of Section 112's enablement requirement. In particular, the Examiner's assertion that the above guidance is not available (provided in the specification or known in the art) is factually incorrect.

Foremost, the Examiner's rejection fails to account for the fact that alpha-amylases, including the alpha-amylase family of which SEQ ID NO:4 is a member of, are some of the most well-characterized enzymes known in the art. SEQ ID NO:4 is a member of the family known as the Termamyl-like alpha-amylases. See the specification at page 6. The Examiner is respectfully directed to the disclosure of WO 96/23874 (corresponding to issued U.S. patent 5,989,169), which is representative of the skill and knowledge in the art for the Termamyl-like alpha-amylase at the time of the invention. WO 96/23874 provides a detailed description of information as to which amino acids should be conserved, which amino acids can be altered and detailed information of the ways in which the protein's structure relates to its function. In particular, WO 96/23874 provides a description of the three dimensional crystal structure of an alpha-amylase representative of SEQ ID NO:4. See WO 96/23874 at Appendix 1. WO 96/23874 also provides a description of the domains found in the structure of the Termamyl-like alpha-amylases, which SEQ ID NO:4 is a member of, in particular, the A, B and C domains, and which amino acids make up the domains. See WO 96/23874 at page 10 to page 14. WO 96/23874 further provides detailed information on the location of the important calcium and sodium binding sites and which amino acids are responsible for the structure of the calcium and sodium binding sites (page 12-

13), as well as the location of the substrate binding site and which amino acids are involved in the interactions with the substrate (page 13). Thus, contrary to the Examiner's allegation, residues and structures which are important to the structure of SEQ ID NO:4 and related sequences having at least 70% identity and which are critical for maintaining the alpha-amylase functionality are clearly known in the art.

WO 96/23874 further provides detailed guidance for which amino acids can be changed to alter the properties of the alpha-amylases, such as, calcium dependency, activity at higher or lower pH, thermostability, cleavage pattern, and thus the specification provides guidance as to which amino acids can be altered without disrupting the alpha-amylase activity. See, e.g., WO 96/23874 at page 30-44.

In addition to this information, the following patent documents evidence the very high level of skill in the art as applied to alpha-amylases, including SEQ ID NO:4, and provide guidance for alterations which encompass the entire sequence of Termamyl-like alpha-amylases. See, e.g., WO 96/23873, WO 91/00353, FR 2,676,456, EP 285 123, EP 525 610, WO 94/02597, WO 94/18314. Again, the Termamyl-like alpha-amylase family, which encompasses alpha-amylases having at least 70% identity to SEQ ID NO:4, are some of the most well-characterized enzymes known in the art. In fact, almost every position has been investigated for its suitability for alteration or its inappropriateness for alteration. This evidence contradicts the Examiner's allegations concerning the relevant skill level, and in particular, the incorrect statements the Examiner made that there is no guidance as to the general tolerance of the enzyme for modification, which amino acids can be altered, and which amino acids are important for preserving the activity of the enzyme.

The Examiner's assertion that the specification or the art lacks guidance for a rationale and predictable scheme for a rationale and predictable scheme for modifying any amino acid residue of any alpha-amylase with an expectation of obtaining the desired biological function is also not factually accurate. In particular, the Examiner's conclusion substantially underestimates the level of skill in the art at the time of the invention. The specification references techniques known in the art to produce and identify functional variants, such as, by site directed mutagenesis and random mutagenesis techniques. See the specification at page 19-23. Using such techniques, it was routine in the art, as of the filing of the present application, to rapidly generate and screen for thousands of alpha-amylase variants using the assays disclosed in the specification. In this regard, the Examiner's allegation that the production and screening of numerous variants is undue may have been true many years ago, however, it was certainly not

true as of the time of the invention.

The Examiner is also directed to information that was known in the art at the time of the invention. In particular, WO 96/23874 teaches how to produce additional members of the claimed alpha-amylase variant genus using the three dimensional structure of a representative alpha-amylase. See WO 96/23874 at, e.g., Example 1-4. In this regard, the United States Patent and Trademark Office has already acknowledged the ability of an artisan to carry these tasks out, as evidenced by issued U.S. Patent No. 5,989,169.

In addition to these "rationale and predictable scheme for modifying any amino acid residue of any alpha-amylase with an expectation of obtaining the desired biological function," the Examiner is also directed to directed evolution protocols available in the art at the time of the invention. Such technology available to the artisan at the time of the invention included the gene shuffling protocols of Stemmer (see, e.g., U.S. Patent No. 6,365,408) and other gene diversity protocols, which permit an artisan to rapidly generate virtually all of the possible variants of a sequence, and certainly, alpha-amylases having 70% identity to SEQ ID NO:4. Thus, although the Examiner has not explained why an artisan even needs to produce and test virtually infinite possibilities, such production and testing was routine in the art at the time of the invention.

Thus, appropriate guidance is provided in the specification and in the art for a rationale and predictable scheme for modifying any amino acid residue of any alpha-amylase with an expectation of obtaining the desired biological function, i.e., alpha-amylase activity. At most, the skilled artisan would have to make and test variants using an assay for functional alpha-amylase activity (alpha-amylase starch-degrading activity) to determine if the alteration(s) disrupts the alpha-amylase activity. Thus, the Examiner has applied a state of art that is not accurately reflective of the state of the art at the time when the invention was made.

The claims have also now been amended to recite that the variants have alpha-amylase activity, and thus, inoperative alpha-amylases would also not fall within the scope of the claims. Nevertheless, with respect to potential inoperative embodiment, the Examiner has also misapplied the law in that the Examiner's rejection improperly implies that potential inoperative embodiments (e.g., from additional alterations not tested) renders the claimed invention non-enabled. The fact that inoperative embodiments may be encompassed by the claims is not enough, by itself, to show non-enablement, as clearly set forth in the Federal Circuit's decision in *Atlas Powder Co. v. E. I. DuPont De Nemours & Co.*, 750 F.2d 1569 (Fed. Cir. 1984). Moreover, the presence of inoperative embodiments is not problem for the skilled artisan, as such embodiments are simply

eliminated by the screening process. Indeed, if desired, the artisan would essentially never even encounter these inoperative embodiments as they are eliminated in the screening process.

Thus, the enablement rejection is maintained because the Examiner has applied an inappropriate state of the art in that the Examiner fails to give consideration to the evidence provided in the specification, the high level of skill in the applicable art, and to the fact that the alpha-amylase art is one of the most well characterized proteins known, with considerable knowledge about the structure of these enzymes, what changes can be made, and what amino acids are important for preserving the activity of the enzyme.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

#### IV. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,



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Jason I. Garbell, Reg. No. 44,116  
Novozymes North America, Inc.  
500 Fifth Avenue, Suite 1600  
New York, NY 10110  
(212)840-0097

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